



TITLE:

# The Behaviors of Acyl-DL-Lysine for Enzyme Action

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Arsono groups. *p*- and *m*-Aminophenylarsonic acid results in the formation of the corresponding phenylenediarsonic acid with less yield of 24% and 13% respectively.

As the case of variously substituted 2-amino-4-hydroxyphenylarsonic acid and 3-nitro-4-aminophenylarsonic acid are converted to the corresponding phenylenediarsonic acids with the yield of 34% and 66%.

It is therefore, conceivable that hydroxyl and nitro groups in the para or ortho position to diazo group facilitate the Bart reaction, while these in the meta position impede the reaction.

Furthermore it seems to illustrate that the strong beneficial influence of *o*-nitro or hydroxyl group overcome the hampering effect of *m*-arsono group.

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### 32. Study on the Aromatic Stibonic Acid. (IV)

*Risaburo Nakai, Hajime Tomono and Tatsuo Azuma.*

Primary aromatic amines can be converted into the corresponding stibonic acids by the Bart reaction or its modification, which involves the interaction of a diazonium salt with a freshly prepared sodium antimonite and alkali.

The preparation of phenyl stibonic acids attained to the yield of 35-40%. For the study of the effect of acetamino group on yield, three isomers of acetamino aniline were prepared. The reaction with the *p*-acetamino aniline, obtained by the reduction of *p*-nitroacetanilid, resulted in the formation of the corresponding *p*-acetaminophenylstibonic acid with the yield of 26%, while the monoacetyl compound derived from *p*-phenylenediamine by acetylation to no effect. The *o*-, and *m*-compounds synthesized by the reduction of nitroacetanilid was converted into the corresponding stibonic acids in the yield of 13% and 8% respectively. A comparison of the yield denotes that an acetamino group impedes the stibonation by the Bart reaction and the hampering effect increases in the order of *p*, *o*, and *m* position to the diazo group.

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### 33. The Behaviors of Acyl-DL-Lysine for Enzyme Action.

*Senji Utzino and Toshio Yoneya.*

Only  $\alpha$ -chloroacetyl derivative was hydrolyzed from  $\alpha$ -formyl,  $\alpha$ -acetyl,  $\alpha$ -chloroacetyl, and  $\alpha$ -benzoyl derivatives of  $\epsilon$ -benzoyl-DL-lysine at pH 7 by crude aqueous extract of hog kidney. The rate of hydrolysis was very slow in comparison with that of monoaminomonocarboxylic acids and amounted to 50% of the theory after 45 hours. The substrates were not attacked at all by the beef pancreas enzyme.

The results of hydrolysis of the substrates by crude mouse kidney or liver extracts which exhibit usually the powerful action of acylase, were the same as those by hog kidney extract;  $\alpha$ -chloroacetylated lysine derivative was only susceptible. In view of the increase of acidity (50 per cent splitting), the stereochemical specificity of acylase, and the lack of susceptibility of  $\epsilon$ -benzoylamino-*n*-caproic acid, no hydrolysis of the  $\epsilon$ -benzoylamino group is expected and the bond where the hydrolysis took place is presumed as to be the part uniting the  $\alpha$ -chloroacetyl group and the L-isomer. This deduction agrees with the report given by Greenstein et al. as  $\epsilon$ -chloroacetyl amino-*n*-caproic acid and chloroacetyl- $\beta$ -alanine show a perfect resistance and the rate of hydrolysis of  $\epsilon$ -carbobenzoxy- $\alpha$ -chloroacetyl- and  $\alpha$ ,  $\epsilon$ -dichloroacetyl-DL-lysine are very slow.  $\epsilon$ -Benzoylamino-*n*-caproic acid,  $\epsilon$ -benzoylamino-DL- $\alpha$ -bromocaproic acid or  $\alpha$ -formyl,  $\alpha$ -acetyl,  $\alpha$ -chloroacetyl or  $\alpha$ -benzoyl derivatives of  $\epsilon$ -benzoyl-DL-lysine were incubated with aniline in the presence of ficin (milky sap of the fig tree). Only with  $\alpha$ -benzoyl derivative  $\alpha$ ,  $\epsilon$ -dibenzoyl-L-lysine anilide precipitates out quantitatively and  $\alpha$ ,  $\epsilon$ -dibenzoyl-D-lysine remains in the solution.

Thus this asymmetric synthesis of the anilide has been employed successfully in the resolution of DL-lysine. DL-Lysine or its  $\epsilon$ -benzoyl derivative was converted to the  $\alpha$ ,  $\epsilon$ -dibenzoyl-DL-lysine. Its sodium salt in 0.1 M citrate buffer solution (pH 4.5) was kept at 37° for 24 hours or more with aniline in the presence of ficin. The separated anilide of L-derivative was refluxed with 6 N KCl for 10 hours. After removal of benzoic acid, the filtrate was concentrated in vacuo to a thick syrup. The residue was taken up in a small amount of hot 100% alcohol and acetone and was added. The precipitate was analytically pure L-lysine dihydrochloride (87 per cent; m. p. 201-202°,  $(\alpha)_D^{23} = +15.5^\circ$  (3% in H<sub>2</sub>O); N; calculated, 12.8, found 12.6. The filtrate from the enzymatic synthesis of L-anilide was heated to boiling to coagulate the proteins, and the filtrate was concentrated in vacuo. After acidification to Congo red, the  $\alpha$ ,  $\epsilon$ -dibenzoyl-D-lysine separated. In the same manner as the L-anilide the precipitate was hydrolyzed with 6 N HCl and produced D-lysine dihydrochloride with m. p. 201-202°,  $(\alpha)_D^{23} = -15.6^\circ$  (3% in H<sub>2</sub>O) and 76% yield (N; calculated 12.8, found 12.7).

### 34. Studies on the Manufacture of Diastase. (III)

*Hideo Katagiri, Toyozo Shibutani and Teruhisa Mugibayashi.*

In the previous papers,<sup>1)</sup> we have determined several conditions for patent No. 126296 and the experimental results obtained after these conditions are given in this report.